CHARITÉ
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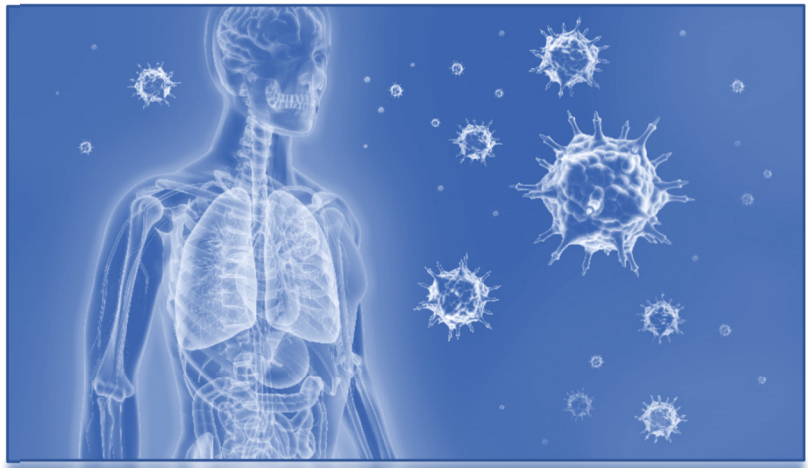
Autumn Meeting 2019

– section cell biology –
of the
Deutsche Gesellschaft für Pneumologie und Beatmungsmedizin e.V.

DGP



CHARITÉ
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Dear colleagues,

we are pleased to welcome you to the **Autumn Meeting 2019** of the DGP section cell biology, which is taking place at the **Humboldt Graduate School** on **November 29th and 30th**.

Scientists and physicians are going to discuss novel scientific and clinical achievements and future research directions, particularly providing a forum for interaction and networking of young scientists.

This meeting has been certified by the Berlin Chamber of Physicians with CME credits.

We hope you enjoy the conference and your stay in Berlin.

With kind regards,

Dr.
Christoph Tabeling

Prof. Dr.
Martin Witzenrath
(Scientific director)

Friday, November 29

13.00 - 13.30 **Welcome Reception**

13.30 - 13.45 **Opening**

13.45 - 14.45 **Session 1: Asthma & Allergic Airway Inflammation**

The cytokine response of circulating immune cells to rhinovirus is reduced in severe asthma
Kaschin Jamal Jameel/Bochum

Altered inflammatory phenotype in obese C57BL/6 mice in a model of house dust mite-induced airway inflammation
Sarah Miethe/Marburg

Antibiotic use during pregnancy increases murine offspring asthma severity in a dose dependent manner
Moumen Alhasan/Berlin

Maternal asthma is associated with persistent changes in allergic offspring antibody glycosylation
Elisa B. Sodemann/Berlin

14.45 – 16.10 **Session 2: COPD and Lung Cancer**

Physiological role of the olfactory receptor OR2AT4 in alveolar macrophages
Daniel Weidinger/Bochum

Role of the PD-1/PD-L1 axis in the development of COPD
Felix Ritzmann/Homburg

Long non-coding transcripts in pneumonia and COPD exacerbation – the BioInflame study
Bernd Schmeck/Marburg

Surface proteome of plasma extracellular vesicles as biomarkers for pneumonia and acute exacerbation of COPD
Bernd Schmeck/Marburg

Preclinical efficacy of a GPR4 antagonist in a murine emphysema-exacerbation model
Annalisa Addante/Wuppertal

Chitinases as potential targets for lung cancer therapy
Anna Brichkina/Marburg

16.10 – 16.40 **Coffee break**

16.40 – 18.15 **Session 3: Cystic Fibrosis, Phages and Vaccines**

Comparison of efficacy of functional rescue of F508del CFTR by approved CFTR modulators
Constanze Vitzthum/Berlin

Inhibition of TRPM4 in airway epithelial cells leads to reduced inflammation and host defense
Ahmed Mahdy/Homburg

Flagellin modulates the differentiation of 3D bronchospheres
Richard Sprott/Homburg

Biofilm reduction by lytic phages
Magdalena Bürkle/Berlin

Morphological and technical aspects affecting phage stability during nebulization
Wibke Groenewald/Berlin

Codon pair deoptimization as tool in the development of live-attenuated vaccines
Nicole Groenke/Berlin

Fully synthetic S. pneumoniae serotype 3 tetrasaccharide conjugate vaccines protect mice from pneumonia
Verena Stössel/Berlin

18.15 – 18.45 **Coffee break**

18:45 – 19:30 **Key note lecture**

Role of the innate immune system in adaptation of organisms to their environment
Andreas Diefenbach/Berlin

20.00 – 22.00 **Joint Dinner - Restaurant im Hamburger Bahnhof (by Sarah Wiener)**
Invalidenstraße 50-51

Saturday, November 30

08.00 – 09.00 **Session 4: Poster Discussions**

Role of the Tie2 agonist Vasculotide in murine Staphylococcus aureus pneumonia
Kevin A. Braun/Berlin

Sputum analysis by chipcytometry following LPS inhalation challenge
Saskia Carstensen/Hannover

Paralysis of pulmonary immunity after stroke by neurohumoral mechanisms
Sandra Jagdmann/Berlin

Ventilator-induced lung injury increases susceptibility of mice to Pseudomonas aeruginosa pneumonia
Chunjiang Tan/Berlin

Lung purinoreceptor activation triggers ventilator induced brain injury
Adrian González-López/Berlin

Defective surfactant biosynthesis and ultrastructural abnormalities of alveolar type 2 cells in pulmonary fibrosis of conditional Nedd4-2$^{-/-}$ mice
Dominik Leitz/Berlin

Inhibition of profibrotic signaling in primary fibroblasts from patients with idiopathic pulmonary fibrosis (IPF) by sirtuin-1/-2-inhibitor sirtinol
Julia V. Zlotkowski/Gießen

Identification of SLC26A9 chloride channel activators by high-throughput screening
Anita Balázs/Berlin

Novel phenotypes of airway solitary neuroendocrine cells and their plasticity in Skn-1a/Pou2f3- knockout mice
Wafaa Mahmoud/Gießen

Analysis of impact and effect mechanism of VacA on DC and their allergen-specific interaction with T cells
Alexandros Contoyannis/Essen

09.00 – 09.30 **Coffee break**

09.30 – 11.20 **Session 5: Pneumonia**

Lung tissue dissociation and viable single cell preparation for single-cell RNA-sequencing – first steps towards a murine pneumonia lung atlas
Peter Pennitz/Berlin

IGF-1R deficiency exacerbates pneumococcal pneumonia
Matthias Felten/Berlin

Neutrophil extracellular traps (NETs) are detrimental in severe p neumococcal pneumonia. A potential therapeutical role for adrenomedullin
Luiz G. T. Alves/Berlin

Pseudomonas aeruginosa flagellin and UGP are determinants of bacterial virulence in an ex vivo model of lung infection
Sven Cleeves/Hannover

Pneumococcal acidification as a virulence mechanism for barrier disruption in human lungs
Diana Fatykhova/Berlin

Delineation of crosstalk-dependent gene expression between human alveolar epithelial type II cells and macrophages upon Influenza A virus infection
Katja Hönzke/Berlin

Mitochondrial calcium flux is decisive for survival of pneumolysin induced cell death
Iris von Wunsch Teruel/Berlin

Therapeutic targeting of myeloid-derived suppressor cells alleviates bacterial superinfection of viral pneumonia
Ling Yao/Berlin

11.20 – 11.50 **Coffee break**

11.50 – 12.30 **Session 6: Ventilator-Induced Lung Injury**

In vivo and in vitro analysis of liquid shear stress on alveolar epithelial cells
Jasmin Matuszak/Berlin

Inflammation impairs circadian rhythms in alveolar epithelial cells
Sebastian Ferencik/Berlin

The post VILI period: a murine model for long-term effects of ventilator-induced lung injury
Matthias Felten/Berlin

12.30 – 13.35 **Session 7: Lung Fibrosis and Pulmonary Hypertension**

Development of pulmonary fibrosis in conditional Nedd4-2 deficient mice
Julia Duerr/Berlin

CXCR4 promotes in vitro and in vivo epithelial cell proliferation, epithelial-mesenchymal crosstalk and fibrosis
Benedikt Jäger/Hannover

Increased susceptibility to gammaherpesvirus-induced lung fibrosis of transgenic mice with conditional overexpression of the ER stress-factor Chop in alveolar epithelium
Mohamed Smaida/Gießen

Fibrogenesis in the murine SP-C deficient lung – Structural air space re-organization meets metabolic macrophage dysregulation
Jannik Ruwisch/Hannover

The role of primary cilia in pulmonary hypertension
Philip D. Solymosi/Berlin

13.35 – 13.45 **Closing remarks**

Abstracts

Session 1: Asthma & Allergic Airway Inflammation

The cytokine response of circulating immune cells to rhinovirus is reduced in severe asthma

Kaschin Jamal Jameel[1], Sarah Yanik[1], Eike Bülthoff[1], Faisal Yusuf[1], Birte Struck[1], Simon Rhode[1], Kai Pfeifer[1], Juliane Kronsbein[1], Andrea Koch[2], Matthias Tenbusch[3], Jürgen Knobloch[1]

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Introduction: Exacerbations trigger the progression of severe asthma and are often caused by airway infections with human rhinovirus (HRV). Asthma subjects are more susceptible to viral infections than healthy subjects. This suggests defects in the immune response to HRV. In response to HRV infections, circulating immune cells become recruited to and activated in the infected lung tissue and in the draining lymph nodes.
Hypothesis: The activation of circulating immune cells in response to HRV is impaired in severe asthma and is influenced by Typ2 inflammation.
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from 34 non-smokers with severe asthma (SA) and 19 healthy never-smokers (NS), cultivated and infected with HRV (strain 16) at MOI 0.1 and 1.0. After 24h and 7d cytokines (activity markers) were measured by ELISA. Baseline-normalized data were compared between NS and SA and were analyzed for spearman correlation with total IgE (n=31 SA) and with markers for Typ2 inflammation: blood eosinophils (n=32 SA), serum-periostin (n=29 SA), and FeNO (n=25 SA).
Results: Baseline IL1β was increased and baseline IFNγ, IL6, IL8 and TNFα were reduced in SA vs. NS. HRV induced CCL2, CCL5, IFNα, IL1β, IL6 and TNFα after 24h and 7d and IFNγ and IL8 after 7d in NS. The HRV-induced responses of all these cytokines except IFNγ were reduced in SA vs. NS. The IL6 response was lower in SA with <300 than in SA with ≥300 eosinophils/µl blood. In SA, HRV-induced IFNα or IL1β or CCL2 correlated negatively to FeNO or periostin or both, respectively.
Conclusions: The cytokine response to HRV is reduced in PBMCs of severe asthma. This suggests systemic immune defects resulting in an impaired activation of circulating immune cells after recruitment to the infected tissue. This molecular pathology might be influenced by the intensity of Typ2 inflammation. The data can explain the impaired infection defense and the increased susceptibility to viral infections in asthma.

Altered inflammatory phenotype in obese C57BL/6 mice in a model of house dust mite-induced airway inflammation

Miethe S.[1], Potaczek D.P.[1], Pauck K.[1], Hagner-Benes S.[1], Garn H.[1]

[1]Philipps University of Marburg, Institute of Laboratory Medicine and Pathobiochemistry – Molecular Diagnostics, Marburg, Germany

Introduction: The so-called obesity-associated asthma phenotype is characterized by atopy, late-onset, and female predominance. However, the pathomechanistic relationships between asthma and obesity are not fully elucidated. Since both diseases are characterized by the presence of chronic systemic inflammatory processes, we pursue the hypothesis that local inflammatory processes in the lung and thus the susceptibility to develop respiratory obstruction and bronchial hyperreactivity are enhanced or modified with respect to the inflammatory phenotype by inflammatory mechanisms of the obese fatty tissue.
Methods: We used BALB/c and C57BL/6 mice in which metabolic changes were induced by a continuous high-fat diet (HFD) over several weeks starting at 4 weeks of life. At 16 weeks, asthma-like inflammatory processes were induced by exposure to house dust mite (HDM) extracts at intervals of one week in parallel to continuing HFD. A mixed inflammatory phenotype model was chosen as asthma model. Weight gain and metabolic markers were assessed over time and lungs and bronchoalveolar lavages (BAL) were analyzed for inflammatory cell composition and molecular markers by FACS and proteome profiler cytokine array.
Results: In animals of both strains with normal diet, HDM exposure resulted in an equal increase of eosinophils and neutrophils in lung and BAL as intended. In BALB/c mice, HFD did not lead to an obvious increase in body weight, although metabolic changes such as increased blood levels of cholesterol, HDL, LDL and triglycerides were present. Further, no changes in the induced asthma phenotype between the HFD/HDM group and the control diet/HDM group were detected. In contrast, comparable differences in the above-mentioned metabolic parameters in response to HFD were associated with a significant weight increase in mice of the C57BL/6 strain. Moreover, HDM exposure resulted in a shift in the composition of inflammatory cells in the HFD/HDM group with absolute higher numbers of BAL eosinophils. Further effects of the HFD were detected in the BAL of C57BL/6 mice using an proteome profiler cytokine array, such as increase in the proinflammatory cytokine TNFα and the proinflammatory adipokines leptin and resistin, but also in the antiinflammaotory cytokine IL-10. Deeper FACS analyses revealed a trend in higher numbers of IL-5$^+$ and IL-17$^+$ CD4$^+$ lung cells reflecting additional impacts of inflammatory fatty tissue mechanisms on the lung.
Conclusions: Among the two strains analyzed, a continuous high fat diet resulted only in C57BL/6 mice in obvious signs of obesity. This was paralleled by changes in the inflammatory phenotype in the lung in response to HDM exposure. The underlying mechanisms of these observations are currently under further investigation which includes also the fascinating question why BALB/c mice behave completely different with respect to weight gain and lung inflammatory phenotype development.

Antibiotic use during pregnancy increases murine offspring asthma severity in a dose dependent manner

Moumen Alhasan[1,2], Alissa M Cait[3], Markus M Heimesaat[1], Michael Blaut[4], Robert Klopfleisch[5], Thomas M Conlon[6], Ali Ö Yildirim[6], Elisa B Sodemann[1], Stefan Bereswill[1], Melanie L Conrad[1,2]

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Introduction: The use of antibiotics during pregnancy is associated with increased allergic asthma risk in the offspring, and given that approximately 25% of pregnant women are prescribed antibiotics; it is important to understand the mechanisms contributing to this phenomenon. Currently, there are no studies that directly test this association experimentally. Our objective was to develop a mouse model in which antibiotic treatment during pregnancy results in increased offspring asthma susceptibility.
Methods: Pregnant mice were treated daily from gestation day 8 to 17 with an oral solution of the antibiotic vancomycin, and three concentrations were tested. At weaning, offspring were subjected to an adjuvant-free experimental asthma protocol using ovalbumin as an allergen. A kinetic analysis of the gut microbiota was performed in mothers and offspring with samples collected from five different time points; short chain fatty acids were also analyzed in allergic offspring.
Results: We found that maternal antibiotic treatment during pregnancy was associated with increased offspring asthma severity in a dose dependent manner. Furthermore, maternal vancomycin treatment during pregnancy caused marked changes in the gut microbiota composition in both dams and pups at several different time points. The increased asthma severity and intestinal microbiota changes in pups were also associated with significantly decreased cecal short chain fatty acid concentrations.
Conclusion: Consistent with the "Developmental Origins Hypothesis", our results confirm that exposure to antibiotics during pregnancy shapes the neonatal intestinal environment and increases offspring allergic lung inflammation.

Maternal asthma is associated with persistent changes in allergic offspring antibody glycosylation

Elisa B. Sodemann[1], Sabrina Dähling[2], Robert Klopfleisch[3], Ekaterina Boiarina[4], Didier Cataldo[6], Moumen Alhasan[1], Ali Önder Yildirim[7], Martin Witzenrath[4,5,8], Christoph Tabeling[4,5,9], Melanie L. Conrad[1,10]

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Introduction: Maternal asthma during pregnancy is considered an environmental risk factor for asthma development in children. Immunoglobulin G (IgG) antibodies that are transferred from the mother to the fetus are known to act in a pro- or anti-inflammatory manner depending on their glycosylation status. Using a mouse model, this study examined how maternal allergic airway inflammation during pregnancy influenced offspring experimental asthma severity, as well as maternal and offspring IgG antibody glycosylation patterns. The effects of maternal and offspring exposure to the same or different allergens was also tested.
Methods: Female mice were either sham sensitized, or sensitized to casein (CAS) or ovalbumin (OVA) before mating. Then, allergic lung inflammation was induced in pregnant dams via aerosol allergen challenge. After weaning, pups were subjected to an experimental asthma protocol using OVA. Asn-297 IgG glycosylation was analyzed in maternal and offspring serum.

Results: When mothers/offspring were sensitized to the same allergen (OVA), several aspects of offspring experimental asthma were more severe when compared to allergic offspring from sham treated mothers. This was evidenced by altered antibody concentrations, increased BAL inflammatory cell influx and decreased lung tissue and lymph node FoxP3 cell numbers. When mothers/offspring were sensitized to different allergens (CAS-OVA) this phenotype was no longer observed. Maternal serum from allergic mothers had significantly higher levels of pro-inflammatory IgG1, evidenced by decreased galactosylation and sialylation at the Asn-297 glycosylation site. These same patterns were reflected in the serum of adult allergic offspring.
Conclusions: We observed a strong association between maternal experimental asthma during pregnancy, offspring airway inflammation and IgG glycosylation patterns in mothers and their offspring. IgG glycosylation is not a standard measurement in the clinical setting; we argue that it may provide valuable information for patient assessment and be an important parameter to include in future clinical studies.

Session 2: COPD and Lung Cancer

Physiological role of the olfactory receptor OR2AT4 in alveolar macrophages

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Introduction: New therapeutic options are required to stop the disease progression of COPD and severe asthma, particularly in phenotypes with neutrophilic inflammation. Alveolar macrophages (AMs) might be a suitable target cell as they play a central role in these obstructive lung disease phenotypes. The understanding of olfactory receptors (ORs), G-protein-coupled receptors that are expressed in many cell types beyond the olfactory epithelium, is still in its infancy. Recent research has suggested them as molecular therapeutic targets in several diseases including NSCLC. Moreover, Ca-dependent signaling and phagocytosis in AM are dysregulated in obstructive lung diseases.
In this project, we investigate human primary AM for expression of ORs and try to understand if OR activity might be linked to the molecular pathology of obstructive lung diseases. This would enable us to decipher its possible role as therapeutic targets.

Methods: AM were isolated from the bronchoalveolar lavage of patients. The expression of ORs was analyzed on mRNA- and protein-level using RT-PCR, Westernblot and Immunocytochemical staining. Calcium Imaging was used for the investigation of intracellular calcium levels and inhibitor experiments. The phagocytosis activity was analyzed using "Flouresbrites PlainYG 0,5 Micron Microspheres" in combination with FACS.
Results: In this early phase of the project, patients were not classified by disease. The receptor OR2AT4 was detected on AM at protein- but not mRNA-level. Immunocytochemical staining revealed OR2AT4 localization in the plasma membrane. Stimulation of AM with the OR2AT4 ligand Sandalore increased intracellular calcium levels in a concentration-dependent manner. Oxyphenylon, a competitive antagonist of OR2AT4, blocked the Sandalore-induced increase in intracellular calcium concentration. Sandalore partially blocked the phagocytosis activity of AM at high concentrations.
Conclusions: AM carry functional OR2AT4 but do not perform OR2AT4 *de-novo* synthesis. OR2AT4 is linked directly to Ca-dependent signaling pathways. OR2AT4 activity interferes with the phagocytosis activity of AM. These data provide first indication for OR2AT4 as a molecular therapeutic target in obstructive lung diseases.

Role of the PD-1/PD-L1 axis in the development of COPD

Felix Ritzmann[1], Giovanna Vella[1], Annette Lis[2], Adrian Angenendt[2], Christian Herr[1], Thomas Tschernig[3], Robert Bals[1], Christoph Beisswenger[1]

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Background: Smoking is the main cause for the development of the chronic obstructive pulmonary disease (COPD). Bacterial pathogens (e.g. Nontypeable *Haemophilus influenzae* (NTHi)) are frequently found in lungs of stable COPD patients. Antibodies that specifically block the receptor programmed death 1 (PD-1) have demonstrated efficacy as therapeutic agents for non-small cell lung cancer.
Methods: Mice were chronically exposed to CS or NTHi and treated with an antibody targeting PD-1 or an isotype antibody. Concentrations of PD-1 and PD-L1 were measured in serum collected from stabile COPD patients and during acute exacerbations (AECOPD) and in BAL fluids of stable patients.
Results: Chronic exposure of mice to CS or NTHi associated with an increased expression of PD-1 and PD-L1 in lung tissue. Anti-PD-1 treatment decreased lung damage and neutrophilic inflammation. PD-L1 concentrations correlated positively with PD-1 concentrations in serum and BAL fluids. The ratio of PD-1 to PD-L1 in BAL fluids correlated with disease severity (FEV1 predicted, GOLD III/IV).
Conclusions: The PD-1/PD-L1 axis is involved in the development of inflammation and tissue destruction in COPD. Inflammation-induced activation of the PD-1 pathway may contribute to disease progression.

Long non-coding transcripts in pneumonia and COPD exacerbation – the BioInflame study

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Introduction: Acute infections of the lower respiratory tract like community-acquired pneumonia (CAP) and acute exacerbations of chronic obstructive pulmonary disease (AECOPD) constitute the third most frequent cause of death worldwide. Urgent medical needs are early diagnosis and differential diagnosis as well as profound pathophysiological insights. Chronic obstructive pulmonary disease (COPD) is a poorly reversible condition characterized by airflow limitation and decrease of lung function. Community acquired pneumonia (CAP) is clinically defined by a sudden onset of severe illness that is accompanied by signs of lower respiratory tract infection, fever, cough and dyspnoea. Long non-coding RNAs (lncRNAs) and long non-coding intergenic RNAs (lincRNAs) are a newly emerging class of regulatory RNAs in the cell that can be involved in a multitude of processes e.g. epigenetic regulation and transcriptional regulation. In this regard, they are potential regulators of gene expression. Their involvement in CAP remains to be investigated.
Methods: We investigated the PBMC RNA profile of 5 healthy donors, 6 pneumonia patients and 6 COPD patients with acute exacerbations by microarray. Differentially expressed genes were subdivided into mRNA and lnc- or lincRNA. We investigated which coding transcript expression patterns correlate with the expression of a given lnc- or lincRNA across all three conditions by pearson correlation. Correlation was defined as $r > 0.7$.

Results: From a total of 540 differentially expressed lnc and lincRNAs between CAP, AECOPD and healthy donors, we found RNAs that were down-regulated with disease, such as LOC100996286. The pool of transcript that correlated strongly with LOC100996286 included e.g. LRRC14 and TRAF5, and showed a strong pathway enrichment with GO terms T cell activation (p=3.03*10^-12), T cell differentiation (p=2.46*10^-11) and lymphocyte differentiation (p=4.92*10^-12). Up-regulated non-coding RNAs included ENSG00000237326, which strongly correlated with genes such as ANKRD33 and CEP104. The GO term that was most strongly enriched among the genes correlating with ENSG00000237326 was myeloid cell homeostasis (p=1.31*10^-6).
Conclusions: Correlation of coding and non-coding RNAs might indicate co-regulation, which might yield to the establishment of interdependent biomarker networks.

Surface proteome of plasma extracellular vesicles as biomarkers for pneumonia and acute exacerbation of COPD

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Introduction: Community-acquired pneumonia (CAP) and acute exacerbations of chronic obstructive pulmonary disease (AECOPD) represent a major burden of morbidity and mortality and their differential diagnosis is critical. A potential source of relevant accessible biomarkers are blood-borne small extracellular vesicles (sEVs).

Methods: We performed an EV Array to find proteins on plasma sEVs that are differentially expressed and possibly allow the differential diagnosis between CAP and AECOPD. Plasma samples from 21 healthy subjects, 24 CAP and 10 AECOPD patients were analyzed. The EV Array contained 40 antibodies to capture sEVs, which were then visualized with a cocktail of biotin-conjugated CD9, CD63 and CD81 antibodies.
Results: We detected significant differences in the protein decoration of sEVs between healthy subjects and CAP or AECOPD patients. We found CD45 and CD28 to be the best discrimination markers between CAP and AECOPD in ROC analyses with an area under the curve of >0.92. Additional ensemble feature selection revealed the possibility to distinguish between CAP and AECOPD even if the CAP patient had COPD, with a panel of CD45, CD28, CTLA4, TNF-R-II and CD16.
Conclusion: The discrimination of sEV-associated proteins is a minimally-invasive method with potential to discriminate between CAP and AECOPD patients.

Preclinical Efficacy of a GPR4 Antagonist in a Murine Emphysema-Exacerbation Model

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This abstract is funded by: BAYER AG

The abstract has been published in the American Journal of Respiratory and Critical Care Medicine 2019;199:A3782.

Rationale: Chronic obstructive pulmonary disease (COPD) is the most common smoking related disease. Frequent exacerbations are considered the most important determinant of disease progression. In COPD, airway acidification is a common phenomenon associated with decreased antibacterial activity and persistent bacterial colonization. GPR4 is a proton sensing G protein-coupled receptor (GPCR) belonging to a family of three closely related receptors including GPR4, OGR1 (GPR65), and TDAG8 (GPR68). GPR4 is highly expressed on endothelial cells. Activated at low pH (pH<7.0) through the protonation of histidine residues of the receptors, GPR4 induces a signaling cascade via coupling to Gs/cAMP formation and serves as an important interface between airway acidification and inflammation. Here we present the characterization of a short-term mouse model that mimics several of the pathophysiological features observed in bacterial COPD exacerbations in humans. Additionally, we report that antagonism of GPR4 by a small molecule antagonist (Miltz, Velcicky et al. 2017) rescues some of these manifestations including lung permeability, inflammation, mucus hypersecretion, airway remodeling and proteases induction.

Methods: Mice received porcine pancreatic elastase (pPE, 0.4U) intratracheally (i.t.) at day 0 to induce emphysema. After 10 days to allow resolution of acute inflammation, lipopolysaccharide (LPS, 2mg/kg) was administrated i.t. to mimic acute bacterial exacerbation. Treatment with the GPR4 inhibitor started 1 day later (twice daily for 4 days, 100mg/kg, per os (p.o.), a dose at which only GPR4 was inhibited as corroborated by Panlabs). To test the validity of the model, measurements of pulmonary function were performed.
Results: Here we show that our emphysema-exacerbation model recapitulates some of the hallmark features of human COPD bacterial exacerbation e.g. small airway remodeling, increased mucus production, increased lung permeability and edema, increased cytokine, proteases production and significant airway obstruction (FEV100ms/FVC was measured). In this emphysema-exacerbation model, we demonstrate that a described GPR4 antagonist significantly reduced lung edema and permeability. Additionally, treatment with the GPR4 antagonist significantly reduced the white blood cells influx in BALF and plasma. Pro-inflammatory cytokines were examined by the means of multiplex cytokine panel, revealing potent anti-inflammatory functions of the compound. Interestingly, the production of detrimental mucin MUC5AC and the expression of proteases involved in pathological matrix remodeling (such as MMP9 and MMP12) were also significantly decreased in response to the treatment.
Conclusions: In this study we characterized a short-term mouse model of emphysema exacerbation; moreover, we demonstrate that GPR4 inhibition is a promising approach for reducing severity of acute COPD exacerbations.

Am J Respir Crit Care Med 2019;199:A3782.

Chitinases as potential targets for lung cancer therapy

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Lung cancer causes the largest number of cancer-related deaths worldwide. More than 85% of lung cancers are currently classified as non-small-cell lung cancer (NSCLC), with a low predicted 5-year survival rate. New treatment strategies are available; however, responses are typically short-lived.
Therefore, there is a demand for the development of more efficient therapeutic regimes. One of the potentially effective approaches to treat cancer is to target both tumor cells and tumor-stroma components, including vasculature, stromal fibroblasts, and immune cells. To assess novel strategies for lung cancer treatment, we investigated a Kras-driven mouse model of NSCLC and clinical material from the patients with lung adenocarcinoma. We found that tumor progression in mouse models of lung cancer correlated with overproduction of secreted lectins – active chitinases – in blood plasma and in the lung tumor lesions. Similarly, patients with lung adenocarcinoma showed increased chitinase level and activity in blood plasma and greater chitinase staining of fixed cancer specimens. We demonstrated that chitinases were produced by macrophages as one of the sources for chitinases in the lung cancer stroma. We propose that chitinase-dependent macrophage functions play a critical role in driving lung tumorigenesis and that blocking this process could have therapeutic potential.

Session 3: Cystic Fibrosis, Phages and Vaccines

Comparison of efficacy of functional rescue of F508del CFTR by approved CFTR modulators

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Introduction: The F508del CFTR mutation is the most common CF-causing mutation. Up to 90% of CF patients carrying F508del at least on one allele and approximately 50% are homozygous for this mutation. Currently there are three approved CFTR modulators for the treatment of certain CFTR mutations available: 1) CFTR potentiator ivacaftor for gating and residual function CFTR mutations, 2) CFTR corrector lumacaftor in combination with ivacaftor for CF patients homozygous for F508del CFTR, and 3) CFTR corrector tezacaftor in combination with ivacaftor for CF patients with at least one copy of F508del CFTR. However, the relative effectiveness of these CFTR modulators is not tested so far. The aim of this study therefore was to investigate the efficacy of the functional rescue of F508del CFTR in cystic fibrosis bronchial epithelial cells (CFBE) by the clinical relevant CFTR modulators ivacaftor, lumacaftor and tezacaftor (single and/or combination treatment).

Methods: To achieve this goal, CFTR-mediated currents were electrophysiologically assessed in Ussing chambers on either wildtype CFTR or F508del CFTR CFBE cells grown on permeable supports at liquid/liquid interface. 48 h prior the Ussing chamber measurements vehicle (0.1% DMSO) and/or compounds (1 µM ivacaftor, 5 µM lumacaftor, 5 µM tezacaftor) were added to the culture media. In addition, F508del CFBE cells were incubation 48 h at 27°C to induce low temperature rescue of F508del CFTR. In Ussing chamber experiments forskolin (1 µM) and IBMX (100 µM) were applied to induce cAMP activation of CFTR. To further activate CFTR ivacaftor (5 µM) was applied followed by the application of the CFTR channel blocker CFTRinh-172 (20 µM).
Results: Ongoing studies indicate that all three tested CFTR modulators activate WT and F508del CFTR in CFBE cells. Lumacaftor/ivacaftor more effective than tezacaftor/ivacaftor and lumacaftor alone followed by ivacaftor alone. The observed currents induced by CFTR modulators are sensitive to CFTRihn-172. The results of this ongoing study will be presented at the meeting.
Conclusions: We predict this study will provide important information on the relative effectiveness of functional rescue of F508del CFTR, that may contribute to an optimized use of CFTR modulators in patients with CF.

Inhibition of TRPM4 in airway epithelial cells leads to reduced inflammation and host defense

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Introduction: Calcium is an important component of many signaling pathways. The TRP-family of receptors belong to a group of transmembrane channels that are essential players for the calcium homeostasis inside the cytosol and endoplasmic reticulum. TRPM4 is a member of the Melastatin-like transient receptor potential channel family and a calcium-regulated potassium-sodium channel. Airway epithelial cells play an important structural and functional role to protect the lung from inhaled pathogens and particles.
The present study will show the influence of the TRPM4 channel on host response and inflammation in airway epithelial cells after the inhibition or knockdown of the TRPM4 channel.
Methods: NCI-H292 cell were cultured under standard conditions (5% CO_2, 95% humidity, 37°C). For the experiments the cells were seeded into standard cell culture plates or 12-well transwell-plates. The knockout of TRPM4 was achieved by the chemical inhibitor 9-Phenantrol or transfection with an shRNA-containing plasmid against TRPM4. In case of shRNA we used a plasmid containing a non-sense hairpin insert as a control. The cell viability was determined using standard LDH-Assay. The cells were stimulated with heat inactivated *P.aeruginosa* PAO1 or LPS. To determine bacterial killing the cells were stimulated with $1*10^3$ CFU of life *P. aerug.* PAO1. The gene expression for IL6, TNF-α, hBD-1, hBD-2, and MIP-2α was determined by qRT-PCR, cytokines were either measured by standard ELISA or Luminex bead-based techniques (both R&D-Systems).

Results: The inhibition of TRPM4 by 9-Phenantrol or shRNA leads to a strongly decreased secretion of IL-6, TNF-α, and MIP-2α after the stimulation with heat inactivated *P.aerug.* while the transcription was unchanged when compared to the corresponding controls. The comparison of cytosolic cell fractions with supernatant showed, that the export of IL-6 was inhibited after the inhibition of TRPM4. Bacterial killing was inhibited in a time dependent manner after the inhibition of TRPM4 and correlated with a decreased expression and secretion of the antimicrobial peptide hBD-2.
Conclusions: This study shows that the inhibition of TRPM4, which likely results in a disruption of potassium-sodium-homeostasis and subsequent changes in calcium concentration, leads to a strongly decreased secretion of inflammatory cytokines and expression of the antimicrobial peptide hBD-2 after bacterial stimulation. These mechanisms may play an important role in our understanding of airway-cell biology and inflammatory diseases like CF or COPD.

Flagellin modulates the differentiation of 3D bronchospheres

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Introduction: Chronic respiratory diseases (e.g. cystic fibrosis) are associated with bacterial infections. Toll-like receptor (TLR) ligands recognize bacterial factors, such as flagellin which is expressed by the cystic fibrosis pathogen *Pseudomonas* aeruginosa. Here, we aimed to investigate the effect of flagellin on the differentiation of human 3D bronchospheres.
Methods: Human primary epithelial cells were differentiated to bronchospheres in Matrigel. Cells were incubated with control media or media containing TLR ligands (100 ng/ml of flaggelin, polyI:C, or Pam3CSK4) during the differentiation period. The appearance of lumina and the presence of cilia were determined.
Results: Differentiation of bronchospheres associated with the formation of lumina and cilia within 30 days after seeding. Incubation with flaggelin resulted in a delayed formation of lumina and decreased ciliation compared to control bronchospheres. Incubation with polyI:C did not affect these parameters whereas Pam3CSK4 slightly accelerated the differentiation of bronchospheres.
Conclusions: Our results indicate that activation of TLR-signaling cascades modifies the differentiation of bronchospheres. Additional analysis will show whether exposure to flagellin induces the expression of mucus and goblet cell metaplasia in bronchospheres. These data show that 3D bronchospheres can be used for mechanistic studies replacing animal experiments.

Biofilm reduction by lytic phages

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Introduction: Biofilm formation e.g., on medical devices or mucosa, represents a survival strategy for bacteria to adapt to their environment. Protected by the biofilm structure, bacteria become more tolerant and resistant to antibiotics and host immune defense, which hampers clinical treatment. As a consequence, conventionally used antimicrobials have limited activity and currently there is no fully efficient method to prevent or eradicate biofilms and biofilm-associated infections. Lytic bacteriophages are viruses capable to replicate within their specific bacterial host cell and kill the host by their own release. Furthermore, bacteriophages can carry depolymerizing enzymes that can degrade exopolysaccharides and therefore disrupt bacterial biofilm. This study aims at analyzing phage activity and efficacy to reduce biofilms produced by *Pseudomonas aeruginosa (P. aeruginosa)*. The project is part of Phage4Cure, a BMBF-funded study aiming to use phages against *P. aeruginosa* lung infections.
Methods: Biofilms of *P. aeruginosa* PaO1 (DSM 19880) were grown on 96-well microtiter plates for 24 hours. Phages were added to the wells and the plates were further incubated for 6 or 24 hours. To quantify the biomass of biofilm, crystal violet staining was performed and the absorbance was measured at 595 nm. In total, two different lytic phages on *P. aeruginosa* PaO1 were tested as well as one Pseudomonas phage without PaO1 specificity and one *Escherichia coli (E. coli)* phage. Additionally, all *Pseudomonas* phages were purified according to laboratory standards and tested accordingly.
Results: Six hours after phage application there was a significant reduction of biofilm biomass by one out of two lytic phages. The second lytic phage did not show a biofilm reduction. Interestingly, after 24 hours, PaO1 regrew and no more biomass reduction could be measured. As expected, the *Pseudomonas* phage without PaO1 specificity and the *E. coli* phage did not reduce biofilm. All results were independent from the degree of purification.
Conclusion: Bacteriophages have the potential to degrade biofilm structures. It is important to get a deeper understanding of the interaction of specific bacteriophages and biofilms to conquer biofilm-associated infections in future efficiently.

Morphological and technical aspects affecting phage stability during nebulization

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Introduction: The increasing number of bacterial infections with multidrug resistant (MDR) species is a major threat to public health. A prevalent MDR bacterium in respiratory diseases and airway infections is *Pseudomonas aeruginosa (P. aeruginosa)*. Bacteriophages replicate only in bacteria with high specificity to their host strain. In order to ensure contact with bacterial cells, topical application is the preferred treatment method. To reach the site of infection in the lung, nebulization of pharmaceuticals is a favorable way for access of the medication to its sites of action. The infectivity of phages with different tail lengths after nebulization was tested under various conditions. This project is part of Phage4Cure, a BMBF funded study examining the use of phages against lung infections.
Methods: Phages active against MDR *P. aeruginosa* with different tail morphologies were nebulized with a JET nebulizer. Long-tailed phages *(Siphoviridae),* short-tailed phages *(Podoviridae)* and phages with medium long contractile tails *(Myoviridae)* from the order *Caudovirales* were analyzed. By using the double-agar overlay method, the titers were compared to the stock solution. In the following, three phages (two *Myoviridae* and one Podovirus) were chosen and purified according to laboratory standards. Their stability at different purification levels during aerosolization with a MESH nebulizer was assessed with the loss of titer.
Results: The loss of titer after JET nebulization was not significant in short-tailed phages. Long-tailed phages showed significantly less plaque forming units in the produced aerosol. Variable and phage-dependent titer loss was observed in the phages belonging to the *Myoviridae* family. Regardless of the degree of purity, MESH nebulization caused significant titer reduction in all three phages. Purification resulted in higher titer loss in 1 out of 3 tested phages *(Myoviridae).*
Conclusion: For successful inhalative phage therapy, impact of nebulizer technology, phage tail size and purity on loss of phage titer during nebulization needs to be considered.

Codon pair deoptimization as tool in the development of live-attenuated vaccines

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Introduction: Codon pair deoptimization (CPD) is an innovative strategy that enables fast and highly efficient attenuation of viruses to generate live-attenuated vaccines. CPD is based on recoding viral genes by increasing the number of codon pairs that are statistically underrepresented without changing the amino acid sequence. It is assumed that underrepresented codon pairs reduce translation efficiency, thereby causing virus attenuation. However, the procedure used for CPD extensively increases the number of CpG dinucleotides in recoded sequences. It is, therefore, unknown whether attenuation by CPD is caused by the modified codon pair bias or increased CpG content.
Methods: We have designed and produced a series of recoded influenza A virus genes, in which codon pair bias is modified but CpG frequencies are not, and vice versa. This has enabled us to disentangle codon pair bias from dinucleotide frequencies and to study the recoded influenza A viruses in tissue culture and infected animals. Additionally we analyzed the mechanism underlying the virus attenuation.
Results: We show that attenuation does not depend on the frequency of CpG dinucleotides but on sub-optimal codon pairs in vitro and in vivo. Underrepresented, i.e. non-optimal codon pairs destabilize mRNA and decrease translation efficiency of recoded genes. Consequently, reduced protein production directly causes virus attenuation.
Conclusions: Our study provides the first evidence that, next not only individual single codons, but also codon pairs are major determinants of mRNA stability. Further, this work demonstrates that codon pair bias can be employed to modulate mRNA stability and protein output of synthetic genes not only in vaccine development but also for a broad range of applications in basic research, biotechnology and therapeutic medicine.

Fully synthetic S. pneumoniae serotype 3 tetrasaccharide conjugate vaccines protect mice from pneumonia

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Introduction: Pneumococcal vaccination is regarded worldwide as the most important preventive measure against invasive pneumococcal diseases. However, the currently used capsular polysaccharide vaccine Prevenar13® has only limited efficacy against serotype 3 (ST3) and the manufacturing process is costly and time-consuming. Synthetic carbohydrate-based vaccines could be an alternative. The present study evaluated in vivo the immunogenicity and protectivity of two fully synthetic ST3 tetrasaccharide antigens conjugated to a glycosphingolipid (GSL).
Methods: Female C57BL/6NCrl mice were subcutaneously vaccinated with one of the two ST3[tetra] vaccine candidates on trial days 0 and 35. The positive control was an experimental group vaccinated with Prevenar13® and the negative control was a group treated with a GSL suspension. On day 49, the animals were transnasally infected with S. pneumoniae. The final analyses were performed 48h after infection.
Results: One of the two synthetic anti-ST3 vaccines and Prevenar13® induced a significant adaptive immune response with production of specific antibodies, whereas the second synthetic test candidate stimulated only a moderate antibody response. All immunized animals showed milder clinical symptoms without hypothermia. Although pulmonary elimination of pathogens only showed a trend towards improvement in vaccinated animals compared to unvaccinated controls, vaccination with the respective synthetic vaccine candidate as well as Prevenar13® protected against the development of bacteremia. Despite an unchanged pathogen-induced inflammatory reaction in the respiratory tract, the endo-epithelial barrier function was better in lungs of mice vaccinated with fully synthetic ST3 vaccines.
Conclusion: The data show a protective effect of fully synthetic vaccine candidates against the development of progressive murine serotype 3 pneumococcal pneumonia.

Session 4: Poster Discussions

Role of the Tie2 agonist Vasculotide in murine Staphylococcus aureus pneumonia

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Introduction: Community acquired pneumonia (CAP) is a significant cause of mortality worldwide. Despite adequate antibiotic treatment, severe pneumonia may induce pulmonary endothelial inflammation and hyperpermeability, resulting in life threatening lung failure. Compared to Streptococcus pneumoniae Staphylococcus aureus *(S.a.)* is a less common pathogen in CAP, but associated with a severe course of disease and a high mortality rate. Angiopoietin-1 mediated Tie2-receptor activation reduces inflammation and stabilizes lung endothelial barrier. We have recently shown that the PEGylated (polyethylene glycol) Angiopietin-1 mimic Vasculotide (VT) reduces lung hyperpermeability in murine pneumococcal pneumonia. The aim of our study was to investigate the influence of VT in an *in vivo* model of S.a. infected mice.
Methods: Pulmonary hyperpermeability, immune cell response and bacterial load were quantified in *S.a.* ($1x10^8$ CFU) infected mice (C57BL/6N) treated with VT (500 ng/100 µl) or PBS (100 µl) in a 12 h interval, starting 10 h post infection (p.i.). Additionally, body weight and body temperature measurements were conducted. Preparation, bronchoalveolar lavage (BAL) and analysis were performed at 12 h, 24 h and 48 h p.i.. Human serum albumin (HSA; 1 mg/75 µl) was intravenously injected 1 h before preparation to quantify endothelial permeability using HSA-BAL-fluid/plasma ratio.
Results: Clinical parameters like body weight and body temperature were not affected by VT treatment. There was a tendency towards reduction in pulmonary permeability 24 h p.i. in the VT treated group in comparison to control group, however reduction was not significant. VT did not demonstrate an impact on pulmonary or systemic leucocytes count or bacterial load.
Conclusions: Present results may indicate that VT stabilizes pulmonary barrier function without impacting immune response in murine S.a. pneumonia, similar to its effects observed in murine pneumococcal pneumonia. However, more detailed investigations are necessary in upcoming experiments.

Sputum Analysis by Chipcytometry following LPS Inhalation Challenge

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Introduction: The LPS inhalation challenge in healthy volunteers is a model for standardized inflammatory immune response of the lung leading to an influx of mainly neutrophilic granulocytes. So far, induced sputum is analyzed by differential cell count or flow cytometry having limitations confining sub populations. Here we tested the performance of Chipcytometry after biobanking of sputum cells in the LPS challenge model by an iterative analysis of multiple cellular markers.
Methods: Induced sputum of 10 healthy non-smokers was collected 2-7 days before and 6h after 2µg LPS inhalation (Janssen et al. 2013). We performed a differential cell count by microscopy (M, n=10), flow cytometry (F, n=9) and Chipcytometry analysis (C, n=9). Flow cytometry was performed immediately following sputum processing. Cells were incubated with a CD45, CD14, CD3, CD8 and CD4 antibody panel. For Chipcytometry, cells were loaded onto specific chamber slides (Zellkraftwerk), fixed with formalin and stored at 4 °C for 8-13 months prior to iterative staining with CD14, CD66b, CD3, CD45, HLA-DR, CD8, CD4 to show signal stabilities.
Results: As known from previous studies, LPS induced a clear increase in sputum neutrophils detectable by all 3 methodologies. The percentage of neutrophils in the differential cell count increased from a median of 37% to 79%. Flow cytometry detected an increase from 62 % to 72 % and Chipcytometry (CD66b) from 45% to 73%. At baseline we observed a significant correlation for the percentages of neutrophils between M and C (r=0.97) and F and C (r=0.90). After LPS challenge the coefficients were r=0.97 and r=0.90. The coefficients of reliability at baseline were 0.95 (M vs. C) and 0.71 (F vs. C), and following LPS 0.96 and 0.75, respectively. CD8 and HLA-DR were not reliably detectable by Chipcytometry showing a donor dependent variant stability of signal after that storage period.
Conclusions: LPS inhalation was mainly characterized by the influx of neutrophilic granulocytes which was detectable by Chipcytometry and showed comparable results to flow cytometry and the standard differential count. Despite a longer storage period than currently recommended for sputum samples we observed a good agreement between methodologies. Our data provides further evidence for the suitability of Chipcytometry for antibody-based sputum cell analysis after storage or shipment to a clinical core facility e.g. in multi-center trials overcoming the disadvantages of flow cytometer measurements.

Paralysis of pulmonary immunity after stroke by neurohumoral mechanisms

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Introduction: Stroke is the third leading cause of death in industrial countries. In Germany, 250.000 people suffer from stroke annually (World Health Organization (WHO) 2008). The outcome depends on the occurrence of medical complications. Up to 95% of stroke patients experience medical complications in the first three month after stroke (Orr-Urtreger et al., 1997). Among these, pneumonia is the most frequent, severe complication (Weimar et al., 2002). Recent experimental and clinical data indicate, that central nervous system (CNS) injuries like stroke leads to an overactivation of neurohumoral stress pathways and consequently to temporary inhibition of peripheral immune response and the development of pneumonia. It was demonstrated in a clinical relevant mouse model of cerebral ischemia (middle cerebral artery occlusion model-MCAO) that an increased cholinergic activity after stroke impairs innate anti-bacterial pulmonary immune responses involving the α7 nicotinic acetylcholine receptor (α7nAChR) expressed on alveolar epithelial cells (AECs) and macrophages (AMs).
Functional cell analysis exhibits that the α7nAChR specific agonist PNU282987 and nicotine inhibit ex vivo LPS induced cytokine secretion in AMs isolated from wild-type (WT) mice and also isolated from α7nAChR knockout (KO) mice. These data suggest, that addition to the α7nAChR other nicotinic receptors may be involved in vagus-mediated suppression of pulmonary immune response.
Methods: The role of α2, α5, α7 and α9/10 nAChR in the cholinergic anti-inflammatory pathway after stroke was investigated in an aspiration-induced post-stroke pneumonia model. Therefore, MCAo was performed in nAChR KO mice and WT littermates. Spontaneously developing pneumonia was prevented by short-term antibiotic treatment one day before and on the same day of MCAO. Bronchoscopy-guided application of defined pneumococcal suspension in the bifurcation of the lung of MCAo nAChR KO mice, MCAo WT mice and naïve WT mice was performed on day 3 after stroke. Microbiological, immunological and histological analysis was done on day 4.
Results: Microbiological analysis has shown, that bacterial burden does not differ significantly between MCAo nAChR KO mice and MCAo WT mice. In contrast, naïve WT mice were able to clear induced bacteria. Infarctsize does not differ significantly between WT mice and nAChR KO mice. Cellular composition of lung and spleen of MCAo nAChR KO mice and MCAo WT mice on day 4 showed no significant changes. Naïve WT mice exhibited significantly increased cell counts of macrophages and neutrophils in spleen and interstitial macrophages in lung.
Conclusions: Thus, α2, α5, α7 and α9/10 nAChR do not play a role in an aspiration induced pneumococcal lung infection after stroke.

Ventilator-Induced Lung Injury Increases Susceptibility of Mice to Pseudomonas Aeruginosa Pneumonia

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Introduction: Ventilator-associated pneumonia (VAP) with *Pseudomonas aeruginosa (PsA)* is a major cause of nosocomial infections in intensive care units, leading to increased patient morbidity and mortality. Mechanical ventilation (MV), normally a life-saving intervention, may exacerbate pre-existing lung injury, a process termed ventilator-induced lung injury (VILI). Whether MV and the associated inflammation have an impact on the development of VAP is not clear, which might be partially explained by the lack of suitable experimental models.
Methods: We established a new murine model of VAP to explore the influence of MV on the development of PsA pneumonia. Sedated female C57Bl/6J mice were subjected to MV. VILI was induced by high tidal volume ventilation (HVt: 34ml/kg), and protectively ventilated (LVt; low Vt: 9ml/kg) animals were used as control. After 4h, mice were detached from the ventilator and PsA was instilled via the ventilation tube. PBS treated animals were used as sham-infected control. After infection, sedation was antagonized and the animals were extubated and breathed spontaneously for 24h.Respiratory function was tested at start and end of MV. Lung permeability, inflammatory responses, and bacterial load (CFU - colony forming units) in lung, blood, liver and spleen were analyzed 24h post infection.
Results: HVt MV led to an increased mean airway pressure (Pao) and decreased lung compliance after 4h of MV. Despite increased pulmonary permeability, the inflammatory response was low in sham-infected mice. Opposingly, HVt ventilated and PSA infected mice showed significantly enhanced alveolar-capillary permeability, increased protein and gene-expression of inflammatory mediators, increased lung and blood neutrophil counts compared to infected LVt ventilated mice. Interestingly the pulmonary and extrapulmonary CFU counts were significantly higher in HVt ventilated animals than in LVt controls.
Conclusion: Mice subjected to VILI (HVt MV) before PsA infection were more compromised by ensuing pneumonia than protectively (LVt) ventilated control mice. This novel murine model of PsA-induced VAP shows that MV associated inflammation is involved in the development of pneumonia and may thus enable for investigations on the pathophysiology of VAP.

Lung purinoreceptor activation triggers ventilator induced brain injury

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Introduction: Mechanical ventilation can cause ventilator induced brain injury via afferent vagal signaling and hippocampal neurotransmitter imbalances, putting critically ill patients at risk for cognitive disorders. The initial triggers for vagal signaling during mechanical ventilation are unknown. Our objective was to assess if pulmonary TRPV4 mechanoreceptors and vagal afferent P2X purinergic receptors act as triggers of ventilator induced brain injury.
Methods: Hippocampal dopamine dependent pro-apoptotic signaling was studied to assess whether non-selective ion channel inhibition with lidocaine, P2X-purinoreceptor antagonist isso-PPADS or genetic TPRV4 deficiency would protect against ventilator induced brain injury in mechanically ventilated mice. Beas-2B human lung epithelial cells were submitted to cyclic mechanical stretch in presence or absence of lidocaine and TRPV4 and P2X expression or activation was quantified. Tandem-mass-tag proteomics and fMRI were used to assess the effects of mechanical ventilation on hippocampal activity. TRPV4 levels were measured in lungs from ventilated and non-ventilated patients.
Results: Intratracheal lidocaine, isso-PPADS and *Trpv4* genetic deficiency protected mice against ventilation-induced hippocampal pro-apoptotic signaling. Mechanical stretch in both, Beas-2B cells and wild type mice resulted in TRPV4 activation and reduced *Trpv4* and *P2x* expression. Intratracheal replenishment of ATP in *Trpv4* $^{-/-}$ mice abrogated the protective effect of TPRV4 deficiency. Proteomic analysis suggested disruption of a neurological disorder related network during mechanical ventilation. Ventilated patients showed decreased lung TRPV4 levels compared to non-ventilated patients.
Conclusions: Lung neuronal pan-inhibition abrogates ventilator induced brain injury. The use of specific inhibitors targeting the TRPV4/ATP/P2X signaling axis may represent a novel therapeutic approach for critically ill patients.

Defective surfactant biosynthesis and ultrastructural abnormalities of alveolar type 2 cells in pulmonary fibrosis of conditional *Nedd4-2*$^{-/-}$ mice

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Introduction: Surfactant protein C, produced by AT2 cells, derives from a proprotein that undergoes several posttranslational modifications. Different mutant proSP-C forms leading to trafficking defects have been shown to be associated with familial and sporadic IPF. Nedd4-2 has been described to be implicated in post-translational processing and trafficking of proSP-C. To study the role of Nedd4-2 in SP-C biosynthesis in vivo we studied mice with doxycycline dependent deletion of *Nedd4-2* in the pulmonary epithelium.
Methods: To obtain mice with dox-dependent deletion of Nedd4-2 *(Nedd4-2*$^{-/-}$*)* in respiratory epithelia, CCSP-rtTA/LC-1 mice providing lung-specific rtTA-mediated dox-dependent expression of Cre-recombinase were crossed with *Nedd4-2*$^{fl/fl}$ mice. To test the impact of SP-C mistrafficking we further intercrossed *CCSP-rtTA/LC-1/Nedd4-2*$^{fl/fl}$ mice with *SP-C*$^{-/-}$ mice. Quadruple mutant mice and littermate controls were treated with dox for 3 months starting at the age of 4 weeks. Lung morphology was evaluated by H&E staining. Functional changes were determined by pulmonary function testing using the Flexivent system. To monitor proSP-C processing confocal microscopy and Western analysis were performed. Ultrastructure of AT 2 cells was assessed by electron microscopy and results were confirmed by Western analysis.
Results: Conditional deletion of *Nedd4-2* results in a chronic progressive lunge disease that is characterized by patchy scarring of distal air spaces and restrictive lung physiology. Electron microscopy showed marked changes in AT2 cell morphology with alterations in number and size and ultrastructural organization of lamellar bodies. Confocal microscopy and Western Blots revealed an altered intracellular distribution of proSP-C and abberations in the proSP-C processing profile in AT2 cells consistent with its mistrafficking as a potential mechanism underlying the observed fibrotic lung disease. To test this hypothesis, we intercrossed *Nedd4-2*$^{-/-}$ with *SP-C*$^{-/-}$ mice and mice were assessed for lung function, histology and inflammation. Surprisingly, *SP-C* deletion was not sufficient to rescue fibrotic lung disease in *Nedd4-2*$^{-/-}$ mice.
Conclusions: We conclude that mistrafficking and defective processing of SP-C in AT2 cells in conditional *Nedd4-2*$^{-/-}$ mice is not the main underlying mechanism of the described IPF-like lung disease. Further studies are required to determine the main pathogenetic mechanism of the IPF-like lung disease in *Nedd4-2*$^{-/-}$ mice.

Inhibition of profibrotic signaling in primary fibroblasts from patients with idiopathic pulmonary fibrosis (IPF) by sirtuin-1/-2-inhibitor sirtinol

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Introduction: Class-I and Class-II histone deacetylase (HDAC)-inhibitors have been identified as successful anticancer-agents, and are already FDA-approved drugs for the treatment of various cancers. Moreover, these Zn^{2+} dependent HDAC inhibitors have recently been proven to attenuate fibrotic remodelling *in vitro* and in bleomycin-treated mice *in vivo*. However, much less is known about Class-III-HDAC-/sirtuin-inhibitors. Inhibitors of NAD^+ dependent sirtuins are currently being targeted as potential therapeutic agents for cancer, as sirtuins are discovered to regulate numerous survival-related downstream enzymes by deacetylation of histones and various non-histone proteins. We investigated sirtuin-expression and the therapeutic potential of the Class-III HDAC-inhibitor sirtinol in primary IPF-fibroblasts *in vitro*.
Methods: Lungs from IPF-patients (n=10) and organ donors (controls, n=6) were analyzed for *SIRT1-7* expression by immunohistochemistry (IHC), western blot and RT-PCR. Primary lung fibroblasts from six IPF-patients were incubated for 20h with vehicle (0.1% DMSO) or sirtinol (3.5 µM), followed by assessment of proliferation and mRNA/protein expression analyses for profibrotic and anti-apoptosis genes, as well as for ER stress and apoptosis-markers.
Results: Sirtuin-1 and -2 were robustly overexpressed in fibroblast foci of IPF-lungs, as indicated by IHC studies. In agreement, transcript level for *SIRT1*, *SIRT2*, *SIRT3* and *SIRT5* were upregulated in IPF- versus control fibroblasts. Treatment of IPF-fibroblasts with sirtinol (3.5 µM) for 20h resulted in reduced cell proliferation, diminished STAT3-phosphorylation and in decreased expression of extracellular matrix (ECM)-components. Furthermore, sirtinol decreased the expression of the anti-apoptosis-gene survivin, enhanced p53/p21 expression, and led to induction of pro-apoptotic ER stress involving CHOP transcription factor, in treated IPF-fibroblasts. In contrast, the sirtuin-1 activator resveratrol (90 µM) did not abrogate the profibrotic phenotype of IPF-fibroblasts *in vitro*, and was associated with a marked increased expression of α-SMA, collagen-I, survivin and cyclin D1.
Conclusions: We conclude that sirtuin-1/-2 inhibitor sirtinol is obviously capable of inactivating IPF-fibroblasts independently of the Zn^{2+} dependent Class-I/-II HDACs, which are not affected by sirtuin inhibitors. *Vice versa*, the FDA-approved Class-I/-II HDAC inhibitors eliminate profibrotic, cancer-like signaling independently of the sirtuins, due to lack of specifity for the NAD^+ dependent sirtuins. It can be speculated that this lack of specifity in both cases might be beneficial for the treatment of cancers or fibrotic disease.

Identification of SLC26A9 chloride channel activators by high-throughput screening

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Introduction: The SLC26A9 chloride channel represents a promising candidate to provide apical chloride transport in the absence of functional CFTR, thus circumvent the primary defect in cystic fibrosis. Recent evidence suggests that SLC26A9 Cl^- channel function may be activated therapeutically by compounds that increase translocation of the protein into the apical plasma membrane. To systematically identify therapeutic target genes and lead compounds promoting trafficking of SLC26A9, we aim to perform high-throughput siRNA and chemical library screens.
Methods: We generated CFBE41o- cells with stable expression of HA-tagged SLC26A9. To be able to measure chloride channel function on a high-throughput scale, we developed a live-cell microscopy-based assay using a membrane potential sensitive dye (FLIPR). Following siRNA-mediated knockdown, changes in membrane potential upon chloride channel inhibition by niflumic acid were measured by time-lapse imaging in 96-well format. Intensity time-traces for individual cells were quantified after segmentation and image quality control. Validation of the effect of siRNA silencing on chloride current was tested in Ussing chamber.
Results: Using the FLIPR assay we quantified the baseline and the response upon adding inhibitor. We were able to detect significant differences between fluorescence intensity changes of scrambled and SLC26A9 siRNA silenced cells. Transepithelial short-circuit current measurements showed that SLC26A9 knockdown significantly reduced the basal and the cAMP-stimulated chloride current, as well as it decreased the inhibition by niflumic acid.
Conclusions/Perspectives: We have developed a robust high-throughput assay to monitor SLC26A9 function. Currently we are testing a hypothesis driven siRNA library for SLC26A9 modulator genes. Hits from a primary screen can be readily validated by Ussing chamber. This platform will enable us in the future to identify therapeutic strategies to activate SLC26A9.

Novel phenotypes of airway solitary neuroendocrine cells and their plasticity in *Skn-1a/Pou2f3*- knockout mice

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Introduction: Rare sensory epithelial cell types in the airway gives rise to various types of small cell lung cancer. These cells include cholinergic chemosensory (TRPM5-positive) cells, villin-positive/TRPM5-negative cells and neuroendocrine cells. Their physiological function is still largely unknown, they may play a role in type II-immunity and asthma. The transcription factor Skn-1a/Pou2f3 is required for development of the cholinergic chemosensory cells. In the taste buds, genetic deletion of *Pou2f3* not only leads to absence of TRPM5-positive taste cells (type II taste cells), but also to increase of other (type III) taste cells. We hypothesized that deficiency in *Pou2f3* also might impact the TRPM5-negative cells populations in the airway epithelium, focusing upon neuroendocrine cells.
Methods: The tracheal epithelium from wild-type, *Pou2f3+/-* and *Pou2f3-/-* mice was assessed by immunohistochemistry to identify and quantify the neuroendocrine cells using antibodies against cell type-specific marker proteins and potential effector molecules (PGP9.5, villin and calcitonin gene-related peptide [CGRP] for neuroendocrine, TRPM5 for chemosensory cells, and CXCL13). Rare sensory epithelial cell types were studied by transmission electron microscopy in wild-type and *Pou2f3-/-* mice.
Results: Ultrastructurally, subpopulations of neuroendocrine cells were not distinguished. Immunolabelling, however, showed at least two phenotypes of neuroendocrine cells with the signatures PGP9.5+/CXCL13+ (71%), PGP9.5+/CXCL13- (29%) or CGRP+/CXCL13+ (74%), CGRP+/CXCL13- (22%) and CXCL13+/CGRP- (4.6%).
All of these types significantly increased in number in both *Pou2f3+/-* and *Pou2f3-/-* mice (PGP9.5+ cells/mm² = 361 in +/-, 286 in -/- and 158 in +/+ mice; CGRP+ cells/mm² = 357, 290 and 156; CXCL13+ cells/mm² = 279.5, 235.6 and 117.9, respectively). The percentage of colocalization between neuroendocrine cell-specific markers and CXCL13 chemokine was preserved in *Pou2f3+/-*, *Pou2f3-/-* and wild-type mice (% of PGP9.5+/CXCL13+ = 69, 71 and 71; % of CGRP+/CXCL13+ = 77, 80 and 74; % of CXCL13+/CGRP- = 4.1, 4.5 and 4.6%, respectively). Immunolabelling showed no immunoreactivity to TRPM5 in *Pou2f3-/-* mice and no difference in the number of TRPM5+ cells between *Pou2f3+/-* and wild type mice (219.7 versus 215.7 immunoreactive cells/mm²).
Conclusions: The B lymphocyte attracted chemokine CXCL13 defines two phenotypes of tracheal neuroendocrine cells, pointing towards a potential regulatory function of these cells in bronchial-associated lymphoid tissue formation. Notably, loss of even only one allele of *Pou2f3* leads to hyperplasia of these cells, demonstrating a hitherto unrecognized role of this transcription factor in population dynamics of these cells.

Analysis of impact and effect mechanism of VacA on DC and their allergen-specific interaction with T cells

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Introduction: Helicobacter pylori (H. pylori) is a gram-negative bacteria occurring mostly in human. It is a common cause for gastric ulcera or gastritis. Recent studies demonstrated that, an infection with H. pylori in the childhood has an adverse impact on the development of Asthma. Indeed, prophylactically treatment of mice with H. pylori or its bacterial extract resulted in an attenuated development of an allergic airway inflammation. Studies investigating Vacuolating Cytotxine A (VacA) deficient H. pylori strains suggesting that this pathogenicity factor plays a decisive role in the suppression of the immune system. In this project, we investigate the impact and effect mechanism of VacA on DC and their allergen-specific interaction with T cells. The generated data is intended to show if the modulation of this immunologic key-event is a central mechanism in the VacA mediated immune-suppression.
Methods: Dendritic cells (BMDC) were generated in vitro from murine bone marrow cells. To analyze if VacA affects the activation of DC, naive and allergen-loaded cells were treated with VacA. Furthermore, the effect of VacA on DC which were activated by the bacterial TLR ligand was investigated an compared to DC activated with the viral TLR ligand Poly(I:C).
Results: We were able to show that VacA has a dose-dependent impact on the expression of the costimulatory molecules CD40 and CD86 on naive and allergene loaded DC. Moreover, VacA enhanced the expression of CD86 on naiv DC which were activated by the bacterial Toll-Like-Receptor (TLR) ligand LPS, an effect which wasn`t present on LPS-activated allergen-loaded DC. Unlike the activation with bacterial LPS, the application of VacA to the Poly(I:C) activated DC lead to an upregulated expression of both CD86 and CD40. By using BMDC from genetically modified mice we demonstrated that the observed effects depend on the signaling pathway components MyD88 and Trif (MyD88/Trif -/-), which are most likely activated by TLR (3D/TLR 2, 4, 5 -/-).
Conclusions: The data show that VacA can affect in a TLR-dependent manner allergen-loaded as well as unloaded-naive DC and modulate their TLR mediated activation. The influence on the expression of DC specific cytokines and the resulting consequences in the developing T-cell response will be the objective of future studies.

Session 5: Pneumonia

Lung Tissue Dissociation and Viable Single Cell Preparation for Single-Cell RNA-Sequencing – First Steps towards a Murine Pneumonia Lung Atlas

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Introduction: In pneumonia, lungs harbor a variety of resident and recruited cells participating in immune responses as well as maintaining organ functionality and physiology under stress. Quality and completeness of a potential murine pneumonia atlas depend on the quality and diversity of the single cell suspension input later subjected to single cell RNA sequencing (scRNAseq). Our aim was to establish a protocol meeting major criteria such as to represent as many cell populations as possible with the greatest possible viability and the lowest possible stress reaction.
Methods: Herewith, we adapted available protocols initially established to isolate fragile alveolar epithelial cells, to allow simultaneous isolation of endothelial cells and leukocytes. Dissociation of lung cells from their matrix into a single cell suspension was achieved by enzymatic digestion and mechanical processing, followed by dead cell removal via magnetic cell sorting. Presence, viability and singlet status of cell populations were controlled by flow cytometry.
Results: We confirmed presence of alveolar macrophages, neutrophils, inflammatory macrophages/monocytes, eosinophils, vascular and lymphatic endothelial cells, type 1 and type 2 alveolar epithelial cells, and bronchial epithelial cells. The viability of all cellular populations analyzed were above the mandatory 80% required for single cell partitioning and barcoding, while doublet rates were in the expected range for the individual cell populations. Single cell suspension quality allowed for Gel beads in EMulsion (GEM) generation and barcoding, followed by cDNA amplification and library generation according to the 10x Genomics protocols. Quality checks performed by fragment analysis (HS NGS Fragment Kit, Agilent) were as expected, so sequencing for gene expression analysis can be performed in the near future. Final conclusions with regards to the matrix dissociation steps - necessary to isolate single endothelial and epithelial cells - inducing stress responses that ultimately overwrite the cells' pneumonia signature will be drawn following attentive analyses of sequences libraries.
Conclusion: The generation of a murine pneumonia atlas seems feasible and may ultimately allow for a deeper understanding of inflammatory processes and contributing cellular populations in the lungs upon acute bacterial infection.

IGF-1R deficiency exacerbates pneumococcal pneumonia

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Introduction: Community-acquired pneumonia (CAP) is a significant cause of acute respiratory distress syndrome (ARDS). Streptococcus pneumoniae (SPN) is the most prevalent causal pathogen identified in CAP. Despite appropriate antimicrobial therapy, pneumococcal pneumonia can progress to ARDS due to alveolar-capillary barrier disruption caused by an exaggerated host-pathogen interaction. Treatment of ARDS is mainly supportive and the discovery of new molecular targets is needed. Previous studies have identified IGF1-receptor (IGF-1R) as an important regulator of pulmonary inflammation. In the present study, we aimed to investigate the role of IGF-1R in pneumococcal pneumonia.
Methods: In vivo, IGF-1R-deficient (UBC-CreERT2;Igf1rfl/fl) and control (Igf1rfl/fl) mice were infected with 5x10^6 SPN (PN36). Mice were sacrificed 48h after infection. Pulmonary permeability, local inflammatory response, pulmonary and extra pulmonary bacterial loads were analyzed. In- vitro, alveolar epithelial cells (A549) were stimulated with SPN (MOI 50, 4 hours) and IGF-1R protein expression was quantified.
Results: IGF-1R-deficient mice had significantly increased pulmonary permeability and increased levels of inflammatory cytokines (MCP-1, IL10) in BAL fluid, whereas the numbers of recruited inflammatory cells were not different compared to control animals. Pulmonary bacterial load was significantly higher in IGF-1R-deficient mice and histological analysis confirmed increased alveolar edema and necrosis-score compared to control and PBS sham-infected mice. In-vitro, SPN caused a decrease in IGF-1R protein expression in alveolar epithelial cells.
Conclusion: Our results demonstrate decreased IgF-1R protein expression in alveolar epithelial cells after SPN challenge and increased susceptibility of IGF-1R-deficient mice to SPN infection compared to control mice. These data suggest that IGF-1R plays a protective role in pneumococcal pneumonia and future studies will investigate the mechanisms by which IGF-1R mediates host-pathogen interactions.

Neutrophil extracellular traps (NETs) are detrimental in severe pneumococcal pneumonia. A potential therapeutical role for adrenomedullin

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Introduction: Community-acquired pneumonia (CAP) has a significant health burden worldwide with high mortality rates despite adequate antibiotic treatment. *Streptococcus pneumoniae* (*S.pn.*) is the predominant pathogen causing CAP. It promotes dysregulation of the innate immune system and disruption of the endo-epithelial barrier functions, leading to acute lung injury and edema formation. Upon *S.pn.* infection, neutrophils accumulate in the lungs aiming the control of the pathogen. However, this contributes to an enhanced inflammation of the tissue, deteriorating disease conditions. We have previously demonstrated that the hormone-peptide adrenomedullin (ADM) protects mice against *S.pn.*-induced lung epithelial barrier disruption, pulmonary edema formation and extra-pulmonary organ damage. In this study, we further propose that ADM protects against *S.pn.* infection by affecting the process of NET formation in neutrophils.
Methods: A murine model for severe pneumococcal pneumonia was assessed to clarify the role of NETs in disease progression. For this purpose, we evaluated different strategies aiming degradation of NETs or its inhibition. The mechanistic properties of ADM in the reduction of NET formation were evaluated *in vitro* in human neutrophils by analyzing its effects on ERK activation and ROS generation.
Results: Neutrophils accumulate in the lungs and release NETs after infection with *S.pn.*. NET degradation with DNase attenuates *S.pn.*-induced lung permeability, suggesting a harmful role of NETs for the lung tissue. The use of alternative mechanism targeting NETs-components or inhibiting its production were beneficial to improve animal clinical conditions and the inflammatory state of the lung tissue. ADM treatment led to a significant reduction in NET release, showing a direct effect of the peptide on this process. *In vitro*, ADM suppressed NET production from stimulated neutrophils through a mechanism that involves cAMP production, inhibition of ERK phosphorylation and ROS reduction.
Conclusions: Our results indicate that NETs have a great deleterious role in the pathology of pneumococcal pneumonia and its inhibition is beneficial for the disease outcome. ADM reduces lung injury and extra-pulmonary organ failure by targeting lung epithelial cell integrity and NET formation and is therefore a promising therapeutic strategy in severe pneumonia.

Pseudomonas aeruginosa flagellin and UGP are determinants of bacterial virulence in an *ex vivo* model of lung infection

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Introduction: *Pseudomonas aeruginosa* infections of the respiratory tract pose a major health care problem. Infection is promoted by virulence factors such as the flagellum, lipopolysaccharide (LPS), toxins and proteases, which are recognized by the host immune system and are tightly regulated. To improve our understanding of *P. aeruginosa* infection and adaptation to the host microenvironment, we aimed to characterize the role of i) flagellin and ii) UDP-glucose pyrophosphorylase (UGP), an enzyme required for the synthesis of LPS, in an *ex vivo* infection model.
Methods: Precision-cut lung slices (PCLS) were prepared from human or rat and infected with *P. aeruginosa* PA14 wildtype (wt) or respective mutant strains that either lack (Δ*fliC*) or accumulate flagellin (Δ*aprAlasB*) or lack UGP (Δ*UGP*). Infected PCLS were treated with different concentrations of tobramycin and analysed 6 h and 24 h post-infection (p.i.). Endpoints included bacterial load (CFU), loss of tissue viability and cytokine release using Calcein AM staining and ELISA, respectively.
Results: While the initial attachment of PA14 to the lung tissue was enhanced by the presence of the flagellum, both wt and Δ*fliC* strains were able to colonize the tissue. Analysis of cytokine profiles revealed a strong upregulation of pro-inflammatory mediators such as IL-8 (6-fold), IL-6 (6-fold), TNF-α (20-fold), GM-CSF (25-fold), IL-1β (52-fold) and MIP-3α (19-fold) 24 h p.i. by PA14 wt compared to non-infected controls. Importantly, infection with Δ*fliC* induced 2- to 4-fold lower levels of the respective cytokines than the wt strain, indicating reduced virulence. Without antibiotic treatment PA14 wt caused strong cytotoxicity in rat PCLS, while PA14 Δ*UGP* was significantly less virulent resulting in a 20-fold reduced loss of tissue viability. Concurrently, growth rates of wt and Δ*UGP* strains were similar, thus the observed effects were not caused by lower CFUs. Interestingly, the minimal inhibitory concentration of tobramycin was four times higher for Δ*UGP* than for wt PA14.

Conclusions: In summary, we show here that PCLS as an *ex vivo* infection model are a valuable tool to study the influence of *P. aeruginosa* virulence factors on i) attachment to and colonization of the lung tissue, ii) tissue viability, iii) the host immune response and iv) tolerance to antibiotics within the complex host microenvironment. In particular, this study shows that flagellin-deficient mutants of *P. aeruginosa* were not hindered in their ability to infect human lung tissue ex vivo. However, the magnitude of the resultant immune response depended on the presence of flagellin. Furthermore, our results indicate that a functional bacterial UGP enzyme is required for full virulence in PA14, underlining its potential as a novel target for drug development.

Pneumococcal acidification as a virulence mechanism for barrier disruption in human lungs

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Introduction: *Streptococcus pneumoniae* (*S.pn.*) is the most frequent causative agent of community-acquired pneumonia worldwide. Although endothelial barrier disruption in the alveolus is considered a major pathomechanism contributing to respiratory failure, little is known about the responsible virulence factors triggering the alteration of the junctional organization in the human alveolo-capillary compartment. We observed that *S.pn.* induced degradation of central junctional proteins such as occludin and VE-cadherin is independent of the major virulence factors pneumolysin or H_2O_2 or even of inflammatory cytokine production. However, during infection of human lung tissue, we observed a strong decrease of pH, which led us to an assumption that *S.pn.* may induce barrier disruption by establishing an acidic alveolar microenvironment.
Methods: To test this hypothesis we used living human lung tissue and human umbilical vascular endothelial cells (HUVEC) for challenging with either *S.pn.* or acidic pH. HUVEC permeability was measured by electric cell-substrate impedance sensing and expression of junctional proteins was assessed with western blot and immunofluorescence. Additionally, extracellular metabolome samples were analyzed by ^{1}H-NMR.

Results: Our data demonstrate a pH- and time-dependent reduction of adherens junction VE-cadherin along with significant decrease in cellular resistance indicating gap formation and barrier loss in HUVEC. A similar effect was shown in *ex vivo* cultivated human lung tissue for junctional proteins VE-cadherin and occludin after *S.pn.* infection or mere pH challenge. Necrosis and apoptosis seem not involved in degradation of cell junctions, since neither LDH secretion was observed nor could treatment with apoptosis inhibitor zVAD prevent the loss of VE-cadherin and occludin. Moreover, neutralization of pH by simple HEPES buffering was sufficient to time-dependently restore junctional expression in the alveolo-capillary compartment up to the critical level of pH 6.25. Glucose consumption and subsequent lactate production was identified as a major product of bacterial fermentation, potentially contributing to strong acidification in the human alveolo-capillary compartment.
Conclusions: Our results render acidification caused by *S.pn.* to an important virulence mechanism for barrier regulation independent of classical virulence factors such as pneumolysin, H_2O_2 or inflammatory cytokine production. Moreover, it seems rational to strictly control alveolar pH during infection in the alveolar microenvironment since buffering showed first promising results for restoring the crucial junctions responsible for barrier stability.

Delineation of crosstalk-dependent gene expression between human alveolar epithelial type II cells and macrophages upon Influenza A virus infection

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Introduction: The majority of respiratory infections in humans are caused by Influenza A virus (IAV) and *Streptococcus pneumoniae* (*S. pneumonia*), which lead to severe community-acquried pneumonia, especially in subsequent co-infections. Virus infected primary human alveolar epithelial type II cells (AECII) secret interferons (IFN) as first line of defense to limit viral replication. At the same time, IFN inhibit *S. pneumoniae* induced IL-1β in alveolar macrophages (AM) thereby suppressing protective GM-CSF release of AECII suggesting a cellular and molecular communication mechanism in the human alveolus. Inhibition of IFN receptor associated Tyk2 completely restored the IL-1β/GM-CSF response, contributing to reduction of bacterial growth. Since little is known about the entire communication between major cellular players in the alveolus under infectious conditions we aim in this project to elucidate the crosstalk-dependent gene expression to better understand the underlying cellular interplay between AM and AEC.

Methods: A549 cells, as established and widely used cell line, and human lung tissue explants as well as primary AECII and AM were infected with A/Panama/2007/1999. We than established a comprehensive gene expression map that highlights differences and similarities first between A549 and AECII and secondly between AECII and AM, as well as human lung tissue under resting and infectious conditions.

Results: Comparative analysis of AECII and A549 transcriptional response upon IAV infection revealed a large overlap of induced genes which are strongly enriched in anti-viral pathways but also an enrichment of numerous pathways in AECII being absent in A549 cells e.g. RIG-I-like receptor signaling. In AECII and AM as well as intact lung tissue, IAV infection leads to a broad transcriptional response, while a set of 302 induced genes were shared between all sample types. AECII appear to up-regulate a greater variety of type I interferons and IFNɣ target genes showed a stronger induction upon viral infection than the same genes in AM and HuLu. AECII and AM specific genes showed an enrichment of the gene ontology (GO) term *response to virus* while the terms *cytokine-mediated signalling pathway* and *response to interferon* in the lung tissue indicate the involvement of cellular crosstalk.

Conclusion: Pathway enrichment analysis revealed the importance of cellular crosstalk in response to influenza virus infection. We showed that the A549 cell line is only restricted usable for IAV infection modelling and that a 3D tissue context is mandatory to study cellular communication mechanisms. A greater understanding of how different cell types respond to the infection and how they interact under infection conditions could provide insights into the pathogenesis of the disease.

Mitochondrial calcium flux is decisive for survival of pneumolysin induced cell death

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Introduction: Apoptosis, a form of programmed cell death, serves as a protective mechanism to eliminate infected or damaged cells. During this process, activation of executioner caspases-3/6/7 are considered as a point of no return. As previously shown, *Streptococcus pneumoniae (S.pn.),* induces executioner caspase activation via its pore forming toxin pneumolysin (PLY) in alveolar epithelial cells. Pore formation leads to rapid cytosolic and subsequent mitochondrial calcium influx $[Ca^{2+}]_m$ resulting in mitochondrial fragmentation, loss of membrane potential and ATP production. Based on these findings we hypothesize mitochondria as a crucial hub in the induction of PLY induced cell death, which might be decisive for cellular fate.
Methods: By using quantitative life-cell imaging and machine learning based digital image analysis we investigated A549 and primary type II epithelial cells during a 20-hour PLY challenge. We tracked and analyzed cells individually with regard to survival / death and correlated above mentioned mitochondrial as well as further cellular parameters such as autophagy induction.
Results: Surprisingly, we identified a significant number of cells that, even though they showed clear caspase-3/7 activation and drop down of mitochondrial membrane potential, were able to recover and survive the PLY challenge. The mitochondrial in-depth analysis on a single cell level revealed the $[Ca^{2+}]_m$ amplitude and efflux rate as decisive parameters for survival and death. Consequently, cells where the $[Ca^{2+}]_m$ raised above a certain threshold while the $[Ca^{2+}]_m$ efflux rate was hampered, were unable to recover, leading to a continuous amplification of caspase-3/7 activation and finally morphological signs of cell death.
Conclusions: Life-cell imaging in cellular biology enables quantification of critical cellular parameters on a single cell level and revelation of otherwise hidden phenomena such as survival of cells after induction of executioner caspases. Moreover, mitochondrial $[Ca^{2+}]_m$ seems to be critical for controlling the cellular fate of alveolar epithelial cells under PLY attack, which might serve as useful insight for therapeutic intervention against detrimental toxin attack during *S.pn.* infection.

Therapeutic targeting of myeloid-derived suppressor cells alleviates bacterial superinfection of viral pneumonia

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Introduction: Bacterial superinfection is a leading cause of morbidity and mortality in viral infections, particularly in influenza virus pneumonia. However, the mechanistic basis for the increased susceptibility of virally infected hosts to subsequent bacterial superinfection remains incompletely understood. In this study, we investigated the role of regulatory immune cell populations, myeloid-derived suppressor cells (MDSCs), induced by influenza A virus (IAV) infection in suppressing anti-bacterial immunity.
Methods: We analyzed the pulmonary accumulation of MDSC in the course of IAV-induced pneumonia in mice. We used adoptive cell transfers to investigate the role of IAV-induced pulmonary MDSCs in a mouse model of IAV-*Streptococcus pneumoniae* (SP) superinfection. We further characterized the interaction of lung MDSCs with macrophages during bacterial infection *ex vivo*. We then leveraged the effects of *all-trans* retinoic acid (ATRA) on the differentiation of immature myeloid cells to target MDSCs as a treatment for bacterial superinfection *in vivo*.
Results: IAV infection led to a marked accumulation of granulocytic (PMN-MDSC) and monocytic (Mo-MDSC) MDSCs in the lungs of infected mice with a peak at day eight post infection. Pulmonary MDSCs displayed a suppressive, immature phenotype and exhibited strong immunosuppressive activity on T cells and macrophages. IAV-infected mice were highly susceptible towards a low-dose-SP infection, and pulmonary PMN-MDSC numbers strongly correlated with impaired bacterial clearance. Adoptive transfer of purified pulmonary MDSCs from IAV-infected mice into naïve animals critically increased the recipients' susceptibility towards bacterial infection. Mechanistically, pulmonary MDSCs potently inhibited the production of pro-inflammatory cytokines in infected macrophages. ATRA treatment successfully differentiated MDSCs into non-suppressive mature myeloid cells *in vitro*, reduced pulmonary MDSC accumulation *in vivo*, and significantly improved bacterial clearance in IAV-SP superinfection.
Conclusions: Our findings identify pulmonary MDSCs as critical regulators of antibacterial immunity in the lung and as a promising therapeutic target in severe bacterial superinfections.

Session 6: Ventilator-Induced Lung Injury

In vivo and *in vitro* analysis of liquid shear stress on alveolar epithelial cells

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Introduction: While mechanical ventilation is a mainstay of therapy for the acute respiratory distress syndrome (ARDS), it can simultaneously trigger adverse complications, most prominently ventilator-induced lung injury (VILI). This fatal association necessitates the need for optimized ventilation strategies, and for a better understanding of alveolar dynamics and the mechanisms underlying alveolar epithelial injury in the mechanically ventilated, injured and edematous lung.
In previous studies, we observed a variety of abnormal dynamics (alveolar dyskinesias), but never anatomical collapse and re-opening of alveoli [Mertens et al., Crit Care Med, 2009; Tabuchi et al., Am J Respir Crit Care Med, 2016]. Therefore, we propose that those microscopic observations does not reflect anatomical opening and collapse of alveoli, but rather cyclic shifts of edema fluid into and out of the alveolus as a function of ventilation pressure according to LaPlace´s law. We hypothesized that cyclic flooding of alveoli with edema liquid, termed by us "alveolar tidal flooding" (ATF), rather than anatomical opening-and-collapse promotes the development of VILI by exerting excessive shear stresses upon the alveolar epithelium.
Methods: We tested this concept by direct visualization of alveolar dynamics by lung intravital microscopy (IVM) in a murine model of alveolar edema [Tabuchi et al., J Appl Physiol (1985), 2008]. ATF was analyzed under a range of different ventilation settings with variation of inspiratory plateau pressure (IPP), positive end-expiratory pressure (PEEP), and driving pressure (ΔP). Cellular injury exerted by cyclic movement of liquid was assessed by measuring LDH and cholesterol in the bonchoalveolar lavage fluid. *In vivo* experiments were confirmed by a cell culture model simulating cyclic movement of an air-liquid interface *in vitro*. LDH and cholesterol release from cells was analyzed, and the number of apoptotic and/or injured cells counted.
Results: ATF was detectable in fluid-filled, yet not in control lungs. The frequency of ATF increased with higher IPP at zero PEEP in flooded lungs, but was prevented by high PEEP. LDH and cholesterol were released from epithelial cells both *in vivo* and *in vitro* during ATF.
Conclusions: We identify alveolar tidal flooding as potentially injurious mechanism of mechanical ventilation in edematous lungs. Alveolar tidal flooding was prominent at low PEEP and high IPP, i.e. at high driving pressure (ΔP) and may as such contribute to the clinically established detrimental effects of high ΔP. Increased levels of LDH and cholesterol implicate a potential role of excessive shear stress as a result of cyclic movement of an air-liquid interface on alveolar functionality and VILI.

Inflammation Impairs Circadian Rhythms in Alveolar Epithelial Cells

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Introduction: Recent studies evidenced the circadian rhythm (CR) as important modulator of innate immune response and lung inflammation. In the majority of acute lung injury (ALI) models, alveolar epithelial cells (AECs) are in first line, determining levels of inflammation and barrier disruption. Whether these cells underlie circadian regulations is currently unknown.
Methods: In order to establish a new in vitro model to analyze CRs in alveolar epithelial cells, H441 AECs were transduced with luciferase transporter constructs for core clock genes (*BMAL1::LUC*, *PER2::LUC*). Transduced cells were grown to confluence and synchronized via dexamethasone application and luminescence was recorded for 7 days with a TopCount camera system. In independent experiments, cells were harvested every 4h over 48h and analyzed on gene expression of circadian genes *CLOCK*, *BMAL1*, *PER1*, *PER2*, *CRY1*, *Nr1d1* and *DBP*. To investigate the effect of different inflammatory stimuli on CRs in AECs we employed bacteria toxin Pneumolysin (PLY) and mechanical stretch (MS). After synchronization cells were stimulated with different doses of PLY for 4h and luminescence was recorded as described. Separately cyclic stretch, similar to injurious ventilation experienced during acute lung injury (18%, 0.25Hz, 24h), was applied to H441 cells and clock gene expression was compared with non stretched controls.
Results: H441 cells demonstrated stable circadian rhythms in luciferase activity and gene expression assays. Stimulation with low concentrations of PLY (0.125µg/µL) led to a prolonged phase. Increasing amounts of PLY enhance the phase shift while retaining the amplitude, indicating that the cells are not dying but the rhythmic expression is directly affected by PLY. Only high doses of PLY > 0.75µg/µL lead to a massive phase shift combined with amplitude loss due to cell death. Injurious MS induced significant alterations in mRNA expression of clock genes after 24h. *BMAL1* expression was 5-fold increased whereas the expression levels of *PER2* and *Nr1d1* where 2fold down regulated.
Conclusion: We conclude that H441 alveolar epithelial cells have a robust CR. Inflammatory stimuli experienced during ALI, such as bacterial toxin PLY or injurious ventilation have a strong impact on CRs and may affect inflammatory processes in AECs. A better understanding of circadian rhythms in the lung opens new ways for target-specific chronopharmacologic therapies.

The post VILI period: A Murine Model for Long-Term Effects of Ventilator-Induced Lung injury

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Introduction: For patients with acute respiratory distress syndrome (ARDS) mechanical ventilation (MV) is a life-saving therapy, but it can also exacerbate the pre-existing lung injury. This process, termed as ventilator-induced lung injury (VILI), is characterized by increased pulmonary permeability, inflammation and activation of the innate immune system. Due to experimental setups to date most studies in mice examine the direct effects of inflammation at the end of MV, so little is known about long-term effects of VILI.
Methods: To explore the temporal course of lung injury caused by MV, we established a new murine model of oro-tracheal intubation. Sedated female C57Bl/6J mice were intubated and subjected to MV. VILI was induced by high tidal volume MV (HVt: 34ml/kg). After 4h, mice were detached from the ventilator, sedation was antagonized and the animals were extubated and breathed spontaneously. Non-ventilated (NV) control mice were ventilated for 10min with low tidal volume (9ml/kg) to record baseline parameters. Respiratory function was tested at start and end of MV. Lung permeability, pulmonary and systemic inflammatory response were analyzed at time point (T) 0h, 6h 12h, 24h, 48h and 5 days (d) after ventilation.
Results: HVt MV led initially to increased mean airway pressure and decreased lung compliance (T0, T6) compared to NV controls. At T12 respiratory function recovered and no significant difference between the groups was detected. Pulmonary permeability was slightly increased after HVt MV at T0, but continued rising until 10-fold increase at T48 compared to respective NV controls. At day 5 no significant difference was detectable between the groups. FACS analysis of recruited inflammatory cells in the bronchoalveolar lavage fluid showed significantly higher counts of neutrophils stable over 48h (T0-T48), whereas at T5d no difference to the control group was measured. In blood samples inflammatory monocyte counts in HVt mice showed an early increase (T0) whereas neutrophil counts increased maximal 2-fold at T6 and T12 compared to controls.
Conclusion: This novel model provides a strong tool to analyze long-term effects and recovery of ventilator-induced changes in the lung. Our results indicate that the lung injury induces by HVt MV is further aggravating within the first 48h before recovery at day 5. For the first time, this model demonstrates the dynamics of inflammatory cell recruitment and alveolar permeability over time and may thus enable for further investigations on the pathophysiology of VILI.

Session 7: Lung Fibrosis and Pulmonary Hypertension

Development of pulmonary fibrosis in conditional Nedd4-2 deficient mice

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Idiopathic pulmonary fibrosis (IPF) is a severe lung disease, which is characterized by chronic irreversible progressive fibrosis and a variable clinical course with acute respiratory worsening constituting a primary cause of death in patients. Nedd4-2 is an ubiquitin ligase that is crucial in the regulation of the epithelial sodium channel ENaC and TGFβ signaling which are both known to play important roles in pulmonary inflammatory diseases and fibrosis. Here, we studied mice with conditional deletion of Nedd4-2 in lung epithelial cells of adult mice. Our results show that conditional Nedd4-2$^{-/-}$ mice develop spontaneous chronic progressive fibrotic lung disease with a continuous decline in lung compliance and increasing levels of IL-13, IL-1β and TGFβ and collagen content in the lung. Histology revealed patchy fibrotic lesions in the periphery of the lung with massive deposition of collagen, destruction of the lung parenchyma and signs of histological honeycombing. Micro-CT images of Nedd4-2$^{-/-}$ mice showed reticular opacities, ground glass opacities, traction bronchiectasis and honeycombing up to 4 months after deletion of Nedd4-2. As reported for patients with IPF, we observed an acute deterioration associated with severe weight loss and hypoxia resulting in an overall mortality of 70% at 4 months after deletion of Nedd4-2 was induced.
Our data suggest that this model may be useful to identify pathogenesis-modulating factors underlying e.g. acute exacerbations, to identify new biological pathways in early pathogenesis, and to develop and test novel therapeutic strategies for patients with IPF.

CXCR4 promotes in vitro and in vivo epithelial cell proliferation, epithelial-mesenchymal crosstalk and fibrosis

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Introduction: Idiopathic pulmonary fibrosis is a fatal disease with mean survival time of 3 years. Here we determined the role of CXCR4 on sphere formation of alveolar epithelial cells. CXCR4 is upregulated and crucially involved in TGF-β mediated epithelial mesenchymal transition (EMT).
Objectives: To test in vitro the role of CXCR4 in de novo generation of spheres with human A549 cells w/o IPF fibroblasts and in vivo the effect of CXCR4 overexpression by A549 cells in a xenograft mouse model for IPF.
Methods: hA549 cells were transduced with a lentivirus leading to CXCR4 overexpression and with an all-in-one CRISPR-Cas9 vector leading to CXCR4 knockout. Control hA549 cells and IPF fibroblasts were transduced with a lentivirus leading to eGFP and luciferase overexpression. hA549 cells were cultivated with or without lung fibroblasts in a transwell system in matrigel. Sphere formation was counted by bright field microscopy and cell proliferation quantified by MTT assay after 14 days. Collagen production were quantified in conditioned media of hA549 w/o fibroblast coculture by sircol collagen assay. Three days after intratracheal administration of a dose of bleomycin, NRG mice were intratracheally injected with hA549 CXCR4 OE, hA549 CXCR4 KO cells and hA549 eGFP and luciferase expressing control cells. Bioluminescence signal was detected by IVIS Imaging. After 14 days the lungs were harvested and used for H&E staining.
Results: Immunohistochemistry revealed an impressive increase in CXCR4 expression in IPF. In particular macrophages, lymphocytes and airway epithelial cells expressed CXCR4 in IPF lung tissues. With CXCR4 overexpressing hA549 cells we observed significantly more and larger spheres [$p=0.0018$] and more cell proliferation [$p<0.05$] compared to A549 CXCR4 KO cells. This effect was even more pronounced in the presence of lung fibroblasts. [$p=0.0001$]. In CXCR4 knockout A549 cells sphere formation and cell proliferation was slightly abolished ($p<0.05$). Then injected in vivo CXCR4 overexpression resulted in larger tumor-like cell proliferates and increased fibrosis compared to wildtype or knockout.
Conclusions: Our data indicate a pathogenic role of CXCR4 expression in epithelial-mesenchymal crosstalk and may be an interesting target for new therapeutic developments in IPF.

Increased susceptibility to gammaherpesvirus-induced lung fibrosis of transgenic mice with conditional overexpression of the ER stress-factor Chop in alveolar epithelium

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Introduction: Idiopathic pulmonary fibrosis (IPF) is an age-related interstitial lung disease with fatal outcome. The main characteristic of IPF is ER stress and apoptosis in type-II alveolar epithelial cells (AECII), which in consequence cause chronic epithelial injury and progressive lung fibrosis. We developed double-transgenic homozygous mice expressing the pro-apoptotic ER stress-factor Chop exclusively in AECII by using the Tetracycline-On-system (SP-C rtTA/tetO7-Chop). In response to doxycycline-feeding (Dox+, 28 days), nuclear Chop overexpression in AECII resulted in AECII apoptosis, but not in development of lung fibrosis in Chop overexpressing mice. We thus suggested that the extent of AECII apoptosis in the 'Chop-mice' was not sufficient to cause lung fibrosis, and that 'second hits' such as age, (herpes)virus-infection, ROS-exposure etc. may be required for 'full-blown' AECII-ER stress and 'high-level' AECII apoptosis. Indeed, several studies have suggested that herpesviruses may play a role in IPF-pathogenesis.
Methods: *Chop* transgenic mice were infected after 28 days transgene-induction (Dox+), as well as without transgene induction (Dox-), intranasally with 5×10^4 plaque-forming units of murine gammaherpesvirus-68 (MHV68). Age-matched non-infected (Dox-) and (Dox+) *Chop* transgenic mice served as further controls (altogether 4 mice groups, all male, 16 weeks of age, n=5 per group). At 15 days post infection (d.p.i.), mice were sacrificed and lungs were analysed for histology and gene/protein expression.
Results: Lungs of MHV68-infected (Dox+) *Chop*-mice, but not of infected (Dox-) *Chop*-mice or uninfected (Dox+) and (Dox-) *Chop*-mice, evidently showed thickening of alveolar septae and fibrotic remodelling. In addition, ER stress and strong apoptosis-induction (caspase-9/-3-activation, PARP1-cleavage) was observed in lungs of both MHV68-infected (Dox+) and (Dox-) *Chop*-mice versus controls, presumably due to virus-induced lung injury, but was more pronounced in the infected (Dox+) *Chop*-mice. Interestingly, Chop was not (endogenously) induced in (Dox-) *Chop*-mice in response to MHV68-infection, despite evident virus-induced ER stress! Further, MHV68-infected (Dox+) and (Dox-) *Chop*-mice indicated upregulation of profibrotic protein-expression (Mmp2, Pai-1, p-Smad3) versus controls, but was "biggest" in the infected (Dox+) *Chop*-mice.

Conclusions: We conclude that our transgenic *Chop*-mouse-model represents a susceptible organism with a vulnerable alveolar epithelium prone to develop lung fibrosis in response to herpesvirus-infection. This mouse model resembles human IPF, since IPF-patients have been defined as (genetically) susceptible individuals prone to develop disease with risk factors/second hits.

Fibrogenesis in the murine SP-C deficient lung – Structural air space re-organization meets metabolic macrophage dysregulation

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Introduction: Lung surfactant is a complex mixture of lipids and proteins, which reduces surface tension at the air-water alveolar interface, decreasing the work of breathing and avoiding alveolar collapse. The main function of surfactant protein C (SP-C) is highlighted during compression of the interfacial film, where SP-C provides stability to the lipid film allowing lower surface tension. Moreover, SP-C has been described to counter-act cholesterol deleterious effect on surfactant film stability at the interface. Animal models with lack of SP-C breathe and live normally, but they show higher susceptibility to chronic lung diseases and induced fibrosis.
Methods and Results: A detailed structural analysis of the lung over 60 weeks of age showed an interesting re-organization of air-spaces in the SP-C deficient mice. Lack of SP-C increases the heterogeneity in alveolar volumes towards bigger over-distended alveolar spaces with age, leading to impaired alveolar dynamics compared to control animals. Numbers of cells in BAL are elevated in SP-C deficient mice and electron microscope pictures showed alveolar macrophages (AM) loaded with different vesicles and lamellar body (LB)-like structures. Therefore, we studied the expression of lipid metabolism genes in these cells. To confirm whether SP-C may have an effect on lipid metabolism and degradation in AM, we performed in vitro endocytosis and gene expression studies using the murine alveolar macrophage cell line MH-S. Addition of cholesterol to surfactant lipids increased the endocytosis ratio of these cells and differentially regulates a set of genes involved in cholesterol transport, regulation and metabolism, such as *Abca1*, *Abcg1*, *Npc2*, *Cd36*, *Pparg*, *Lal* and *Dhcr24*. SP-C in combination with cholesterol in surfactant membranes further activates genes related to cholesterol transport and regulation.
Conclusions: In conclusion, SP-C seems to play an important role not only in surfactant activity and alveolar dynamics, but also in lipid homeostasis in alveolar macrophages and the lung.

The role of primary cilia in pulmonary hypertension

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Introduction: Vascular remodeling in pulmonary arterial hypertension (PAH) is characterized by endothelial dysfunction and smooth muscle cell proliferation and hypertrophy leading to an increase in mean pulmonary artery pressure and ultimately heart failure and death. Up to now, the molecular mechanisms of pathogenesis have not been adequately resolved, preventing the development of novel therapies specifically targeting vascular remodeling in PAH. Based on increased expression levels of PDGF-BB and TGF-β in both patients and animal models with PAH, these signaling pathways have been proposed to play a critical role as drivers of lung vascular remodeling.
Among multiple other effects, PDGF-BB and TGF-β cause shortening of the primary cilium – an antenna-like organelle functioning as a flow-sensor and signaling hub on most eukaryotic cells. Loss or shortening of cilia is characteristically associated with cell proliferation and promotes PDGF signaling, thus potentially establishing a positive feedback loop.
Here, we hypothesize that PAH is associated with a loss or shortening of primary cilia in pulmonary artery endothelial (PAECs) and smooth muscle cells (PASMCs) which in turn drives cell proliferation and thus, remodeling in the pulmonary artery wall.
Methods: PAECs and PASMCs from PAH patients and non-PAH donors were exposed *in vitro* to three different characteristic stimuli or mediators, respectively, of PAH, namely PDGF-BB, TGF-β1, or hypoxia (1%). Cells were fixed and stained with antibodies against acetylated α-tubulin, in order to measure the length of primary cilia. In parallel, donor and PAH-SMCs were compared functionally in migration and proliferation assays. Finally, pulmonary artery tissue from PH-patients and donors was fixed and stained to determine the number of primary cilia.
Results: In pulmonary arteries of PAH patients, the number of primary cilia was reduced by 80% as compared to donor lungs. PAH-SMCs were found to have shorter cilia and showed increased migration and proliferation as compared to healthy control cells. Additionally, primary cilia of PAECs, PASMCs, and PAH-SMCs stimulated with PDGF-BB, TGF-β1, or hypoxia were shorter as compared to controls. Lithium, which has been shown to elongate cilia in other cell types, was able to rescue cilia length and reduced migration and proliferation in PAH-SMCs.
Conclusions: Here, we demonstrate that PAH is associated with a reduction of cilia number *in vivo* and in length *in vitro*. Primary cilium loss may promote progression of vascular remodeling as indicated by association of cilium loss and proliferation/migration in PAH-SMCs, and their parallel reversal by lithium. Cilium loss may as such present an important propagator of vascular remodeling in PAH and may present a target for novel therapeutic interventions such as lithium.

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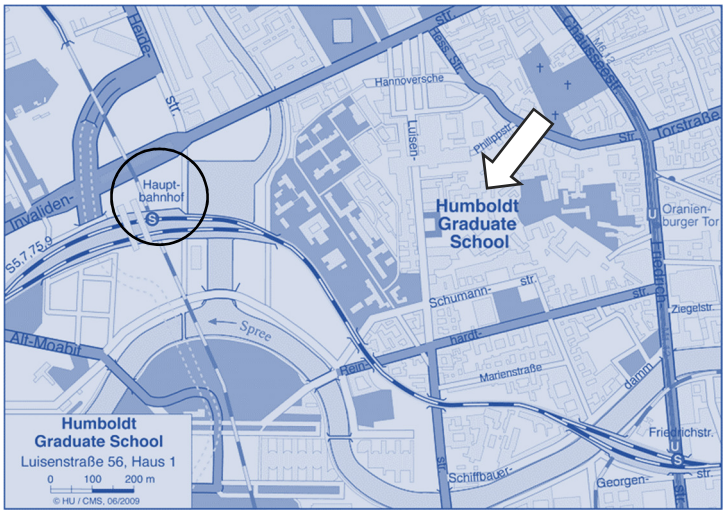

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